Enhanced Activity of the Nucleopolyhedrovirus of the Fall Armyworm (Lepidoptera: Noctuidae) on Bt-Transgenic and Nontransgenic Sweet Corn with a Fluorescent Brightener and a Feeding Stimulant

ROBERT R. FARRAR, JR.,[1] MARTIN SHAPIRO,[2] AND B. MERLE SHEPARD[3]

USDA–ARS, Insect Biocontrol Laboratory, Beltsville, MD 20705

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ABSTRACT The effects of a nutrient-based feeding stimulant, Coax, and a stilbene-based optical brightener, Blankophor P167, on the activity of the nucleopolyhedrovirus of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (SfMNPV) on transgenic sweet corn, *Zea mays* L., expressing a CryIA(b) toxin from *Bacillus thuringiensis* (Berliner), were studied in the laboratory. Both Coax and Blankophor P167 increased virus-induced mortality. The effects of both materials did not differ between transgenic and nontransgenic corn. The greatest increase in virus-induced mortality occurred when Coax and Blankophor P167 were combined. Neither material affected the percentage of larvae killed by the CryIA(b) toxin.

KEY WORDS fall armyworm, nucleopolyhedrovirus, Bt transgenic corn, feeding stimulant, fluorescent brightener

THE FALL ARMYWORM, *Spodoptera frugiperda* (J. E. Smith), is a common pest of corn, *Zea mays* L., in the warmer parts of the Americas (Sparks 1979). It attacks all aboveground parts of the plant. The pest overwinters in southern Florida, where it infests sweet corn annually throughout the growing season (Janes 1973, Foster 1989). Further north, where it does not overwinter, it occurs later in the growing season (Hoffmann et al. 1996).

Transgenic crop plants expressing toxins derived from *Bacillus thuringiensis* (Berliner) (Bt) have become increasingly important as management tools for a variety of lepidopterous and coleopterous pests (Peferoen 1997, Jenkins 1999, Khetan 2001, Kumar 2003). A number of lines of transgenic corn expressing the CryIA(b) δ-endotoxin are resistant to important lepidopterous pests of corn, including the corn earworm, *Helicoverpa zea* (Boddie), and the European corn borer, *Ostrinia nubilalis* (Hübner) (Lynch et al. 1999a, b). However, these lines tend to be less resistant to the fall armyworm (Abel and Pollan 2004, Farrar et al. 2004). Recently, Buntin et al. (2004) reported that field corn expressing a Cry2A(b) toxin suffered less damage than did lines expressing a Cry1A(b) toxin but that pyramiding the two genes did not further improve control.

Nucleopolyhedroviruses (NPVs) are naturally occurring viruses to which many sawflies and caterpillars, including the fall armyworm, are susceptible. Naturally occurring fall armyworm NPV, the *Spodoptera frugiperda* multiply embedded nucleopolyhedrovirus (SfMNPV), can be an important factor in the population dynamics of the fall armyworm in the field (Fuxa 1982, Fuxa and Geaghan 1983). NPVs are used in production agriculture on only a limited basis for a number of reasons, however. These reasons include short residual activity in the field, low potency, and slow speed of kill (Entwistle and Evans 1985, Huber 1986, Adams and McClintock 1991, Hunter-Fujita et al. 1998).

A number of studies have examined the potential use of combining *B. thuringiensis* (usually as preparations of spores and toxin crystals; in a few cases, transgenic plants) with NPVs. No consistent pattern is evident from these studies; interactions between the two pathogens may be synergistic, additive, or antagonistic (reviewed by Farrar et al. 2004). Farrar et al. (2004) found that when fall armyworm larvae reared on foliage of transgenic corn expressing a CryIA(b) toxin or nontransgenic corn were fed equal dosages of occlusion bodies (OBs) of SfMNPV, virus-induced mortality was higher in larvae feeding on transgenic corn. However, when larvae were allowed to feed ad libitum on treated foliage, virus-induced mortality was

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[1] Corresponding author: USDA–ARS-IBL, Bldg. 011A, Rm. 214, BARC West, Beltsville, MD 20705 (e-mail: FarrarR@ba.ars.usda.gov).

[2] Present address: Clemson University, Coastal Research and Education Center, 2700 Savannah Highway, Charleston, SC 29414.

[3] Clemson University, Coastal Research and Education Center, 2700 Savannah Highway, Charleston, SC 29414.

higher on the nontransgenic corn. Higher feeding rates on nontransgenic corn apparently overcame differences in susceptibility of the larvae to SfMNPV.

A number of types of adjuvants designed to improve insect control by NPVs have been tested. Among these adjuvants are derivatives of diaminostilbene disulfonic acid, commonly known as fluorescent brighteners or optical brighteners, which have been shown to reduce the quantity of OBs necessary to kill insects in a number of cases (Shapiro 1995, Farrar and Ridgway 1997, Farrar et al. 1999, Hamm 1999), including SfMNPV against fall armyworm (Hamm and Shapiro 1992, Hamm et al. 1994, Shapiro and Hamm 1999). Brighteners are thought to increase the susceptibility of insects to NPVs by disrupting the peritrophic membrane (Wang and Granados 2000) or by inhibiting sloughing of infected midgut cells (Washburn et al. 1998). In addition, fluorescent brighteners absorb UV light and re-emit the energy as visible light (hence the fluorescence), and as a result, they can also protect NPVs from degradation by sunlight (Shapiro 1992, 1995, Farrar and Ridgway 2000).

Nutrient-based feeding stimulants also have been shown to improve control of lepidopterous larvae by NPVs and *B. thuringiensis* (Farrar and Ridgway 1994, 1995, Farrar et al. 1999). Feeding stimulants developed for lepidopterous larvae are usually composed of vegetable flours, oils, and sugars. They are intended to cause insects to ingest larger amounts of a microbial insecticide and/or ingest it faster than they would in the absence of the feeding stimulant.

This study was undertaken to extend previous results (Farrar et al. 2004) by examining the effects of a fluorescent brightener and a feeding stimulant on the activity of SfMNPV on transgenic and nontransgenic sweet corn. Also in this study, the effect of a feeding stimulant on the efficacy of transgenic foliage alone was examined. Improvement in efficacy of *B. thuringiensis* spore and crystal preparations through the use of feeding stimulants has been widely published (Farrar and Ridgway 1995). However, no information is available on the effect of these materials on insects feeding on transgenic foliage.

Materials and Methods

Insects, Plants, Virus, and Adjuvants. We obtained *S. frugiperda* eggs from stock cultures (Crop Protection and Management Research Laboratory, USDA-ARS, Tifton, GA).

Seeds of transgenic sweet corn, cultivar Attribute, which constitutively expresses the CryIA(b) δ-endotoxin of *B. thuringiensis* ssp. *kurstaki*, and of its nontransgenic isoline, were obtained from Syngenta Seeds (Boise, ID). Plants were grown in 14-cm diameter (1.9 liter) pots, two to four plants per pot, in a greenhouse. A commercial potting medium (Pro Mix BX; Premier Brands, Red Hill, PA) was used. Plants were grown under a temperature of 24 ± 3°C, with the photoperiod supplemented to 16:8 (L:D) h by low-pressure sodium vapor lamps, and were fertilized weekly (Peters Professional 20–20-20; Grace-Sierra, Milpitas, CA). All plants were in the late whorl stage, 6–8 wk old, when used.

SfMNPV was originally isolated from fall armyworm larvae collected from a field in Georgia by J. J. Hamm (USDA-ARS, Tifton, GA). The working stock of this virus was produced by passage through fall armyworm larvae. The virus was applied to the surface of artificial diet (King and Hartley 1985) at a rate of $\approx 10^6$ OBs per 30-ml cup (surface area of ≈ 800 mm^2). Late fourth to early fifth instars were placed individually in cups with treated diet. Larvae that died of viral infection were triturated in distilled water, and the homogenate was filtered through cheesecloth. A hemacytometer was used to count OBs under a phase contrast microscope (×400 magnification).

The feeding stimulant Coax was obtained from *Agro*Solutions (San Marcos, CA). Coax was previously shown to stimulate feeding in the fall armyworm (Farrar and Ridgway 1994) and was commercially available at the time of the study (2003–2004). It is a liquid flowable material containing 35% dry matter.

The fluorescent brightener Blankophor P167 was obtained from Bayer (Rock Hill, SC) as a water-soluble powder. This material has been shown to enhance SfMNPV (Shapiro and Hamm 1999) and was also commercially available at the time of the study.

Excised Foliage Tests. Fall armyworm larvae were reared from hatching through the first stadium on either transgenic or nontransgenic foliage as described previously (Farrar et al. 2004.) Suspensions of SfMNPV (100 OBs/μl) were prepared with and without Coax (1% dry wt:vol). Control treatments of water only and Coax at 1% without virus were also prepared. Joint Venture (Helena Chemical, Memphis, TN), a spreader/sticker marketed for use with biological insecticides, was included in all treatments at 0.125% (vol:vol). Razor blades were used to cut pieces of foliage of 1–2 cm^2 from both types of corn. Pieces of leaves were dipped in the suspensions and allowed to dry and then placed individually in cells of plastic bioassay trays (Bio-BA-128; CD International, Pitman, NJ) with moist filter paper disks (1 cm diameter). Late first instars (showing head capsule slippage) to early second instars were placed individually in cells with foliage. Larvae were placed on the same type of foliage on which they had been reared. Larvae were held at 27 ± 2°C with a photoperiod of 16:8 (L:D), allowed to feed for 48 h, and transferred to new bioassay trays filled with artificial diet (King and Hartley 1985). Mortality was recorded at the time larvae were transferred to diet (early mortality) and again 8 d later (virus-induced mortality). Causes of early mortality were not determined, but differences between corn types were assumed to be related to the δ-endotoxin. Larvae that had died after 8 d and turned dark and liquefied were classified as having been killed by SfMNPV. Twenty-four larvae were included in each treatment combination (corn type and virus/Coax treatment), and the test was replicated five times.

Percentage early mortality was calculated and normalized by arcsine $\sqrt{\%}$ transformation. This variable was subjected to factorial analysis of variance (ANOVA) with corn type, Coax treatment, and their interaction as factors (PROC GLM; SAS Institute 1999.) Percentage virus-induced mortality was calculated and normalized similarly. This variable was analyzed factorially for effects of Coax treatment, corn type, and their interaction. Because no virus-induced mortality occurred on treatments with no virus, these treatments were not included in the latter analysis.

SfMNPV was tested with Blankophor P167 in a manner similar to that for Coax. Suspensions were prepared as before except with 1% (wt:vol) Blankophor P167 instead of Coax. The bioassay and data analyses were the same as in the test of Coax.

The combination of Blankophor P167 and Coax was also tested with SfMNPV. The treatments with 100 OBs/μl and 1% Blankophor P167 killed ≈90% of the larvae. Therefore, to be able to detect possible additional increases in mortality by the combination of Coax and Blankophor P167, the rate of Blankophor P167 was lowered to 0.5%, and in most treatments, the rate of virus was lowered to 50 OBs/μl. The concentration of Coax was maintained at 1%. Treatments included SfMNPV alone and with Coax, with Blankophor P167, and with Coax and Blankophor P167 together. Also included was a treatment with 500 OB/μl alone as a point of reference, with controls of water only and of Coax plus Blankophor P167. The bioassay was similar to previous bioassays except that the number of larvae per treatment was reduced to 16 and the number of replicates was increased to 8.

Early and virus-induced mortality was calculated and normalized as before. Early mortality was analyzed by factorial ANOVA for effects of corn type, Coax, Blankophor P167, and all interactions. Virus-induced mortality was analyzed similarly, but for only those treatments with SfMNPV at 50 OBs/μl. Virus-induced mortality was also analyzed with all virus-containing treatments included. In this analysis, virus and adjuvant treatments were treated as class variables. Data were analyzed by ANOVA for effects of corn type, treatment, and their interaction, and treatment means were separated by the least significant difference (LSD) test.

Potted Plant Tests. Corn plants, grown in a greenhouse as described above, were thinned to two plants per pot. Leaves were trimmed to within ≈10 cm of the stalks so that the whorls could be enclosed in sleeve cages. Sleeve cages were made of slightly elastic knit fabric tubing sold for use as sleeves for wire screen insect cages (BioQuip, Gardena, CA). The tubing was 20 cm across when flat, and it was cut into 38-cm sections to make sleeve cages. A sleeve cage was placed over each plant and secured around the stalk below the whorl with a wire twist tie. Sixteen neonate larvae were placed in each cage, and the cages were closed with twist ties. Infested plants were held in the laboratory at 24 ± 2°C for 72 h.

A spray booth (Research Track Sprayer, model SB8; DeVries Manufacturing, Hollandale, MN) was used to treat potted plants. A single Tee Jet 8002 flat fan nozzle (Spraying Systems, Wheaton, IL), mounted ≈15 cm above the whorls, was used. Assuming that the plants represent one row in a field with 76-cm rows, this system was calibrated to deliver the equivalent of 187 liters/ha (20 gal/acre) at a pressure of 3.52 kg/cm^2 at a speed of 1.92 km/h. The sleeve cages were opened and retracted to below the whorls just before treatment and closed immediately after treatment to prevent escape of larvae.

SfMNPV was applied at a rate equivalent to 2.47×10^{11} OBs/ha with and without Coax at 1% (dry wt:vol). Controls of both corn types were treated with water only or with Coax only. Joint Venture was included in all treatments at 0.125%. One pot of Bt corn and one pot of non-Bt corn were sprayed simultaneously with each treatment. Treated plants were held at 27 ± 2°C for 48 h. Plants were dissected to recover larvae. Surviving larvae were counted, placed on artificial diet, and held at 27 ± 2°C for 8 d, and the number of larvae killed by SfMNPV was recorded. The test was replicated six times. Data from the two plants in each pot were pooled for analysis. Data were analyzed similarly to those from the test of Coax on excised foliage.

SfMNPV was tested on whole plants with Blankophor P167 in a manner similar to that for Coax, except that the rate of SfMNPV was lowered to 2.47×10^{10} OBs/ha because, based on the excised foliage tests, greater increases in mortality were expected from the brightener than from Coax. Suspensions were prepared as before except with 1% (wt:vol) Blankophor P167 instead of Coax. This test was replicated seven times. Statistical analyses were identical to those for the test of Coax.

The combination of Blankophor P167 and Coax was also tested with SfMNPV on whole plants. SfMNPV was applied at a rate of 2.47×10^{10} OBs/ha with and without Coax, Blankophor P167, or the combination, each at 1%. Also included was a treatment with 2.47×10^{11} OBs/ha alone as a point of reference, and controls of water only and of Coax plus Blankophor P167. Because of the larger number of treatments and limited availability of usable plants, not all treatments were tested at the same time. A randomized incomplete block design was used, with each block containing four treatments. One block was set up each week for 14 wk, with each treatment included a total of eight times.

Data were analyzed similarly to those in the test of excised foliage with both Coax and Blankophor P167. If the number of larvae collected from the two plants in a pot was less than five, which occurred in five observations, that observation was not included in the analyses of virus-induced mortality.

Results

Excised Foliage Tests. In the test of Coax, early mortality of larvae (48 h; while they were on foliage) was slightly, although statistically significantly ($P <$ 0.05), higher on the non-Bt corn (Table 1). Coax had no effect on early mortality. Coax, however, significantly increased virus-induced mortality on both

Table 1. Means with SEs and results of statistical analyses of early mortality (after 2 d on foliage) and virus-induced mortality of fall armyworm larvae on Bt and non-Bt corn foliage treated with SfMNPV and/or Coax

Corn	Virus (OB/μl)	Coax (%)	Early mortality (%)	Virus-induced mortality (%)
Non-Bt	0	0	10.83 ± 2.12	[a]
	0	1	10.00 ± 2.83	[a]
	100	0	8.33 ± 2.28	23.30 ± 9.58
	100	1	5.83 ± 2.12	41.64 ± 5.69
Bt	0	0	3.33 ± 1.56	[a]
	0	1	5.83 ± 2.83	[a]
	100	0	5.83 ± 2.83	12.23 ± 2.82
	100	1	13.33 ± 4.82	39.10 ± 6.18
Dependent variable	Independent variable	*F*	df	*P*
Early mortality	Corn type	4.23	1,32	0.0475
	Coax	0.19	1,32	0.6644
	Corn × Coax	1.96	1,32	0.1713
Virus-induced mortality	Corn type	1.36	1,12	0.2658
	Coax	19.03	1,12	0.0009
	Corn × Coax	0.50	1,12	0.4920

[a] Treatments with no virus were not included in the analyses of virus-induced mortality.

types of corn (Table 1). Corn type did not affect virus-induced mortality. There was no virus-induced mortality on either treatment without virus in this or any of the other tests.

In the test of Blankophor P167 on excised foliage, early mortality was slightly higher on treatments with the brightener on both types of corn (Table 2). Blankophor P167 greatly increased virus-induced mortality on both types of corn. Corn type again did not affect virus-induced mortality.

When Coax and Blankophor P167 were combined on excised foliage, early mortality was slightly increased by Coax on both types of corn (Table 3). Virus-induced mortality was higher on non-Bt corn and was increased on both types of corn by Coax and Blankophor P167. A significant interaction of Coax with Blankophor P167 was also seen. When data were analyzed with treatments as a class variable and means separated by LSD, treatment effects were significant ($F = 78.21$; df = 4,63; $P = 0.0001$), whereas the interaction of corn type by treatment was nonsignificant ($F = 0.82$; df = 4,63; $P = 0.5194$). Mortality on the treatment with both Coax and Blankophor P167 was significantly ($P < 0.05$) higher than that on the other treatments, whereas mortalities on the treatments of virus only (lower rate) and virus plus Coax were significantly ($P < 0.05$) lower than those on other treatments.

Potted Plant Tests. Early mortality in the test of Coax on potted corn plants (after 5 d on plants) was higher on the Bt corn but was unaffected by Coax (Table 4). Virus-induced mortality was higher on the non-Bt corn and was increased by Coax on both types of corn.

In the test of Blankophor P167 on potted plants, early mortality was again higher on the Bt corn and was not significantly affected by the brightener (Table 5). Virus-induced mortality was higher on the non-Bt corn but was not significantly affected by Blankophor P167.

Early mortality in the test combining Coax and Blankophor P167 on potted plants was higher on the

Table 2. Means with SEs and results of statistical analyses of early mortality (after 2 d on foliage) and virus-induced mortality of fall armyworm larvae on Bt and non-Bt corn foliage treated with SfMNPV and/or Blankophor P167

Corn	Virus (OB/μl)	P167 (%)	Early mortality(%)	Virus-induced mortality (%)
Non-Bt	0	0	3.33 ± 2.04	[a]
	0	1	6.67 ± 2.83	[a]
	100	0	4.17 ± 2.28	24.7 ± 6.85
	100	1	15.00 ± 3.39	91.59 ± 4.01
Bt	0	0	5.00 ± 3.06	[a]
	0	1	10.83 ± 3.39	[a]
	100	0	1.67 ± 1.02	21.27 ± 4.09
	100	1	6.67 ± 3.63	88.07 ± 3.38
Dependent variable	Independent variable	*F*	df	*P*
Early mortality	Corn type	0.04	1,32	0.8358
	P167	16.35	1,32	0.0003
	Corn × P167	0.11	1,32	0.7369
Virus-induced mortality	Corn type	0.59	1,12	0.4581
	P167	128.85	1,12	0.0001
	Corn × P167	0.07	1,12	0.7925

[a] Treatments with no virus were not included in the analyses of virus-induced mortality.

Table 3. Means with SEs and results of statistical analyses of early mortality (after 2 d on foliage) and virus-induced mortality of fall armyworm larvae on Bt and non-Bt corn foliage treated with SfMNPV and/or Coax and/or Blankophor P167

Corn	Virus (OB/μl)	Coax (%)	P167 (%)	Early mortality (%)	Virus-induced mortality (%)
Non-Bt	0	0	0	3.13 ± 1.67	[a]
	0	1	0.5	7.03 ± 1.84	[a]
	50	0	0	8.59 ± 5.27	22.95 ± 5.52
	50	1	0	15.63 ± 4.72	28.01 ± 3.54
	50	0	0.5	10.16 ± 3.72	67.90 ± 7.31
	50	1	0.5	10.16 ± 4.71	90.10 ± 3.45
	500	0	0	7.03 ± 3.63	52.66 ± 6.73
Bt	0	0	0	4.69 ± 4.56	[a]
	0	1	0.5	9.38 ± 3.34	[a]
	50	0	0	9.38 ± 4.57	7.94 ± 1.99
	50	1	0	16.41 ± 3.11	12.81 ± 2.49
	50	0	0.5	5.47 ± 1.42	57.88 ± 2.96
	50	1	0.5	13.28 ± 3.82	80.13 ± 2.63
	500	0	0	11.72 ± 2.75	47.85 ± 6.37
Dependent variable	Independent variable	F	df	P	
Early mortality	Corn type	2.11	1,97	0.1491	
	Coax	13.13	1,97	0.0005	
	P167	0.39	1,97	0.5324	
	Corn × Coax	0.12	1,97	0.7339	
	Corn × P167	1.96	1,97	0.1649	
	Coax × P167	4.71	1,97	0.0324	
	Corn × Coax × P167	1.23	1,97	0.2706	
Virus-induced mortality[b]	Corn type	22.70	1,49	0.0001	
	Coax	21.56	1,49	0.0001	
	P167	289.45	1,49	0.0001	
	Corn × Coax	0.15	1,49	0.6978	
	Corn × P167	0.74	1,49	0.3932	
	Coax × P167	7.57	1,49	0.0083	
	Corn × Coax × P167	0.26	1,49	0.6147	

[a] Treatments with no virus were not included in the analyses of virus-induced mortality.
[b] Analysis of treatments with 50 OBs/μl SfMNPV only.

Bt corn but was unaffected by Coax or Blankophor P167 or by any interaction (Table 6). Virus-induced mortality was significantly increased by both Coax and Blankophor P167, and the interaction was significant as well. Virus-induced mortality was not significantly affected by corn type in this test. A significant corn type by Coax interaction also was seen. When the data were analyzed with treatments as a class variable and means separated by LSD, treatment affects were significant ($F = 20.67$; df = 4,51; $P = 0.0001$), whereas the treatment by corn interaction was nonsignificant ($F = 1.76$; df = 4,51; $P = 0.1518$). In this analysis, mortality on the treatment with both Coax and Blankophor P167 was significantly ($P < 0.05$) higher than that on the other treatments. The treatment with 2.47×10^{11} OBs/ha had significantly ($P < 0.05$) higher mortality than the remaining treatments and that with 2.47×10^{10} OBs/ha with neither Coax nor Blankophor P167

Table 4. Means with SEs and results of statistical analyses of early mortality (after 5 d on plants) and virus-induced mortality of fall armyworm larvae on potted Bt and non-Bt corn plants treated with SfMNPV and/or Coax

Corn	Virus (OB/ha)	Coax (%)	Early mortality (%)	Virus-induced mortality (%)
Non-Bt	0	0	27.60 ± 8.05	[a]
	0	1	29.69 ± 4.55	[a]
	2.47×10^{11}	0	25.52 ± 8.52	66.06 ± 7.81
	2.47×10^{11}	1	29.17 ± 9.01	83.80 ± 4.27
Bt	0	0	46.87 ± 4.77	[a]
	0	1	51.04 ± 3.67	[a]
	2.47×10^{11}	0	44.27 ± 5.00	45.59 ± 6.23
	2.47×10^{11}	1	54.17 ± 6.59	69.36 ± 9.13
Dependent variable	Independent variable	F	df	P
Early mortality	Corn type	27.14	1,39	0.0001
	Coax	1.89	1,39	0.1769
	Corn × Coax	0.02	1,39	0.9010
Virus-induced mortality	Corn type	6.84	1,15	0.0195
	Coax	9.32	1,15	0.0081
	Corn × Coax	0.03	1,15	0.8660

[a] Treatments with no virus were not included in the analyses of virus-induced mortality.

Table 5. Means with SEs and results of statistical analyses of early mortality (after 5 d on foliage) and virus-induced mortality of fall armyworm larvae on potted Bt and non-Bt corn plants treated with SfMNPV and/or Blankophor P167

Corn	Virus (OB/ha)	P167 (%)	Early mortality (%)	Virus-induced mortality (%)
Non-Bt	0	0	38.54 ± 4.09	[a]
	0	1	42.41 ± 3.40	[a]
	2.47×10^{10}	0	37.50 ± 5.24	42.14 ± 8.74
	2.47×10^{10}	1	35.27 ± 5.09	44.15 ± 4.04
Bt	0	0	56.25 ± 8.32	[a]
	0	1	56.25 ± 4.15	[a]
	2.47×10^{10}	0	50.89 ± 6.39	18.54 ± 5.59
	2.47×10^{10}	1	57.14 ± 7.43	25.24 ± 9.43
Dependent variable	Independent variable	F	df	P
Early mortality	Corn type	18.71	1,45	0.0001
	P167	0.31	1,45	0.5826
	Corn × P167	0.08	1,45	0.7735
Virus-induced mortality	Corn type	7.20	1,18	0.0152
	P167	0.82	1,18	0.3777
	Corn × P167	0.08	1,18	0.7743

[a] Treatments with no virus were not included in the analyses of virus-induced mortality.

had significantly ($P < 0.05$) lower mortality than the other virus treatments.

Discussion

Both Coax and Blankophor P167 may have the potential for use as adjuvants for SfMNPV on both types of sweet corn. Coax increased virus-induced mortality in all tests. Blankophor P167 also increased virus-induced mortality in all tests except in one test on potted plants (Table 5). In that test, mortality also tended to be higher on treatments with Blankophor P167, but the effect was not statistically significant ($P > 0.05$). However, both on excised foliage and on potted plants, the greatest increase in virus-induced mortality occurred when Coax and Blankophor P167 were combined. A significant ($P < 0.05$) statistical interaction between these materials was seen in both tests. Adding both

Table 6. Means with SEs and results of statistical analyses of early mortality (after 5 d on foliage) and virus-induced mortality of fall armyworm larvae on potted Bt and non-Bt corn plants treated with SfMNPV and/or Coax and/or Blankophor P167

Corn	Virus (OB/ha)	Coax (%)	P167 (%)	Early mortality (%)	Virus-induced mortality (%)
Non-Bt	0	0	0	32.42 ± 12.6	[a]
	0	1	1	35.16 ± 8.75	[a]
	2.47×10^{10}	0	0	27.34 ± 7.23	24.21 ± 6.00
	2.47×10^{10}	1	0	30.47 ± 8.61	32.05 ± 6.42
	2.47×10^{10}	0	1	26.56 ± 6.71	43.28 ± 8.59
	2.47×10^{10}	1	1	35.94 ± 9.26	70.21 ± 6.59
	2.47×10^{11}	0	0	41.4 ± 8.55	65.83 ± 7.51
Bt	0	0	0	57.42 ± 8.88	[a]
	0	1	1	57.81 ± 4.42	[a]
	2.47×10^{10}	0	0	65.23 ± 4.78	12.94 ± 3.79
	2.47×10^{10}	1	0	67.58 ± 7.45	32.04 ± 4.80
	2.47×10^{10}	0	1	61.72 ± 8.75	27.35 ± 12.06
	2.47×10^{10}	1	1	69.14 ± 6.69	78.90 ± 4.90
	2.47×10^{11}	0	0	72.66 ± 3.72	45.96 ± 7.91
Dependent variable	Independent variable	F	df	P	
Early mortality	Corn type	38.75	1,91	0.0001	
	Coax	0.04	1,91	0.8367	
	P167	0.02	1,91	0.8924	
	Corn × Coax	0.50	1,91	0.4800	
	Corn × P167	0.25	1,91	0.6203	
	Coax × P167	1.43	1,91	0.2355	
	Corn × Coax × P167	1.30	1,91	0.2579	
Virus-induced mortality[b]	Corn type	2.42	1,38	0.1282	
	Coax	29.01	1,38	0.0001	
	P167	28.13	1,38	0.0001	
	Corn × Coax	4.14	1,38	0.0489	
	Corn × P167	0.47	1,38	0.4960	
	Coax × P167	5.67	1,38	0.0224	
	Corn × Coax × P167	0.43	1,38	0.5147	

[a] Treatments with no virus were not included in the analyses of virus-induced mortality.
[b] Analysis of treatments with 2.47×10^{10} OBs/ha SfMNPV only.

Coax and Blankophor P167 caused an increase in mortality greater than that caused by a 10-fold increase in the rate of virus in both tests. Effects of Coax and Blankophor P167 were similar on both types of corn.

Our results with respect to the fluorescent brightener are consistent with those of Hamm et al. (1994), who obtained increased mortality of fall armyworm larvae collected from field plots of corn treated with SfMNPV when brightener was added. Feeding stimulants were not a part of that study, however. SfMNPV is less potent against its host than are many other NPVs against their homologous hosts, and brighteners usually cause greater enhancement with NPVs of relatively low potency.

Coax apparently had no effect on mortality caused directly by the δ-endotoxin. Early mortality, that which occurred while larvae were feeding on foliage and presumably would be subject to effects of the toxin, was slightly increased by Coax in one test on excised foliage (Table 3). However, this effect occurred on both Bt and non-Bt corn; the interaction of Coax and corn type was not significant ($P > 0.05$). In no other test did Coax affect early mortality. Thus, while nutrient-based feeding stimulants have been shown to improve control of lepidopterous larvae by *B. thuringiensis* spore/crystal preparations (Farrar and Ridgway 1994, 1995), no evidence was found that they might improve the efficacy of Bt transgenic plants in the absence of viruses. Tests of feeding stimulants in other transgenic plant/insect systems would be needed to evaluate the generality of this finding.

Virus-induced mortality was greater on non-Bt corn than on Bt corn in one test on excised foliage (Table 3) and in two tests on potted plants (Tables 4 and 5). In no case was the opposite effect seen. Higher virus-induced mortality on non-Bt corn is consistent with previous results (Farrar et al. 2004) showing that, when larvae were allowed to feed ad libitum on treated foliage, reduced rates of feeding on Bt corn led to less virus-induced mortality.

Early mortality of larvae on Bt corn was higher than that on non-Bt corn in all potted plant tests (Tables 4–6). Early mortality was similar on the two types of corn in the tests on excised foliage, except for a weak effect in one test (Table 1). The difference in these effects between the two groups of tests may be because of the fact that larvae were older at the beginning of the tests on excised foliage, and early mortality occurred over a shorter interval (48 h) in those tests. In the potted plant tests, early mortality was that which occurred over a period of 5 d while larvae were on foliage, beginning at the neonate stage. Before testing on excised foliage, larvae had been reared on the same type of foliage for several days (see Methods and Materials), and so more mortality could have occurred on Bt foliage before the bioassay. Farrar et al. (2004) found that higher mortality occurred in younger larvae when they were reared on Bt foliage from hatching. Other insect species, such as *H. zea*, are more susceptible to the toxins expressed in the Bt foliage used in these tests (Farrar et al. 2004); if similar studies were conducted on such insects, greater effects of corn type would be expected

When Bt-transgenic sweet corn cultivars are planted to control such pests as the European corn borer, additional control measures may be unnecessary. However, when an infestation of the fall armyworm is present, the use of SfMNPV could provide the additional control needed. The use of Coax and Blankophor P167 may improve control and/or allow for reduction in rates of the virus while maintaining control of the fall armyworm on either Bt or non-Bt sweet corn. While our results show that these materials can increase virus-induced mortality in the laboratory, further work is needed to determine if these materials can be of practical use in the field. These results need to be confirmed in the field, and any benefits seen there weighed against costs. Evaluation of SfMNPV on Bt and non-Bt corn in the field is ongoing.

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